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**OAK RIDGE  
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**Water Quality Criteria for Colored Smokes:  
1,4-Diamino-2,3-dihydroanthraquinone**

**FINAL REPORT**

**MARTIN MARIETTA**

**Kowetha A. Davidson  
Patricia S. Hovatter  
Robert H. Ross**

**January 1988**

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19. ABSTRACT (Continue on reverse if necessary and identify by block number) <p>The available data on the environmental fate, aquatic toxicity, and mammalian toxicity of 1,4-diamino-2,3-dihydroanthraquinone (DDA), an anthraquinone dye used in violet-colored smoke grenades, were reviewed. The U.S. Environmental Protection Agency (USEPA) guidelines were used in an attempt to generate water quality criteria for the protection of human health and of aquatic life and its uses.</p> <p>Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. During typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation is released into the aquatic environment. The primary aquatic system receiving these discharges is the Arkansas River and its associated drainages.</p> <p style="text-align: right;">(continued on back)</p>				
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DDA will readily oxidize to 1,4-diaminoanthraquinone (DAA) in air or during combustion of the smoke grenade. The dye is insoluble in water; however, no information is available concerning its transformation or transport in soil, water, and sediments.

No data are available concerning the toxic effects of DDA in aquatic organisms; therefore, a Criterion Maximum Concentration and a Criterion Continuous Concentration cannot be determined. Toxicity studies following the USEPA guidelines are recommended.

Very few data were found on the toxicity of DDA in laboratory animals, and no data were found on the toxicity of the dye in humans. The lethal concentrations  $\times$  time causing 50 percent mortality (LC<sub>50</sub>'s) derived from acute inhalation exposure of seven animal species to violet smoke (80 percent DDA and 20 percent Disperse Red 9), disseminated from grenades, were 206,393 mg·min/m<sup>3</sup> for all rodents (rats and guinea pigs), 160,013 mg·min/m<sup>3</sup> for all nonrodents (monkeys, dogs, rabbits, swine, goats), and 211,205 mg·min/m<sup>3</sup> for all species combined. The LC<sub>50</sub>'s ranged from 39,731 mg·min/m<sup>3</sup> in monkeys to 399,831 mg·min/m<sup>3</sup> in goats. The ultimate toxic component in violet smoke appears to be the combustion or oxidation product, DAA.

DDA is a weak mutagen in the Salmonella Reversion Assay, but the combustion or oxidation product, DAA, is a strong mutagen in the same test. Violet smoke is noncarcinogenic in the SENCAR Mouse Skin Tumor Bioassay.

Because threshold or nonthreshold chronic toxicity data in laboratory animals or humans were not available, a criterion for the protection of humans, using the USEPA guidelines, could not be derived.

**Water Quality Criteria for Colored Smokes:  
1,4-Diamino-2,3-dihydroanthraquinone**

**FINAL REPORT**

**Kowetha A. Davidson  
Patricia S. Hovatter  
Robert H. Ross**

**Chemical Effects Information Task Group  
Information Research and Analysis Section  
Health and Safety Research Division**

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## EXECUTIVE SUMMARY

1,4-Diamino-2,3-dihydroanthraquinone (DDA) is a relatively unstable anthraquinone dye used by the military in M18 violet-colored smoke grenades that are deployed for communication. The dye is used as a chemical intermediate in the dye industry. It is readily prepared by the condensation of ammonia with 1,4-dihydroxy-2,3-dihydroanthraquinone.

The environmental release of DDA may occur during manufacturing, during formulation and loading of violet smoke grenades, or upon detonation of grenades during training and testing operations. Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. The primary aquatic system receiving wastewaters at the arsenal is the Arkansas River and its associated drainages. Prior to the installation of a pollution abatement facility in 1979, contamination to this system from untreated pyrotechnic wastes was reported as significant.

Sufficient data to determine the environmental fate of DDA are lacking. The dye will readily oxidize to 1,4-diaminoanthraquinone (DAA) in air or during combustion of the smoke grenade. DDA is insoluble in water; however, no information is available concerning its transformation or transport in soil, water, and sediments.

Based on a calculated octanol-water partition coefficient of 0.0456, bioaccumulation of DDA should be negligible. No data are available concerning the toxic effects of DDA in aquatic organisms; consequently, neither a Criterion Maximum Concentration nor a Criterion Continuous Concentration can be determined. Toxicity studies required by the U.S. Environmental Protection Agency (USEPA) guidelines are recommended for deriving these criteria.

Very few data were found on the toxicity of DDA in laboratory animals, and no data were found on the toxicity of the dye in humans. Acute inhalation exposure of seven animal species to violet smoke containing 80 percent DDA, disseminated from grenades, produced an overall 36 percent mortality. Toxic signs included respiratory difficulty, gagging, vomiting, wheezing, general weakness, ataxia, and prostration. The  $LC_{50}$ 's ranged from 39,731 mg·min/m<sup>3</sup> in monkeys to 399,831 mg·min/m<sup>3</sup> in goats. The  $LC_{50}$  was 211,205 mg·min/m<sup>3</sup> for all species combined, 206,393 mg·min/m<sup>3</sup> for all rodents (rats and guinea pigs), and 160,013 mg·min/m<sup>3</sup> for all nonrodents combined (monkeys, dogs, rabbits, swine, goats). Although violet smoke mixture contains 20 percent Disperse Red 9, the toxic component appears to be DDA, because, in another test, red smoke (100 percent Disperse Red 9) was shown to be less toxic than violet smoke. In addition, DDA is quantitatively converted to DAA upon detonation of the grenades, indicating that the ultimate toxic component is DAA.

In the Salmonella Reversion Assay, DDA is a weak mutagen, but its combustion or oxidation product, DAA, is a strong mutagen. Violet smoke is noncarcinogenic in the SENCAR Mouse Skin Tumor Bioassay.

Because threshold or nonthreshold chronic toxicity data in laboratory animals or humans are not available, a water quality criterion for the protection of human health, using the USEPA guidelines, cannot be derived.

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## 1. INTRODUCTION

1,4-Diamino-2,3-dihydroanthraquinone (DDA) is a relatively unstable anthraquinone dye used by the military in M18 violet-colored smoke grenades that are deployed for communication. The violet smoke mixture is composed of 80 percent DDA and 20 percent Disperse Red 9 (Cichowicz and Wentzel 1983). DDA is also used as a chemical intermediate in the synthesis of other anthraquinone dyes (Abrahart 1968, as cited in Kitchens et al. 1978; Dacre et al. 1979).

The pyrotechnic composition of colored smoke grenades consists of the dye mixture, an oxidizer, fuel, coolant, and diatomaceous earth as a binder. Each grenade contains approximately 352 g of the dye mixture, which is formulated at Aberdeen Proving Ground, Maryland (Smith and Stewart 1982). The cooling agent is used to prevent excessive decomposition of the organic dye due to heat produced by the fuel. Upon detonation of the grenade, heat from the burning fuel causes the dye to volatilize; the vapor then condenses outside the pyrotechnic, thereby producing smoke. The burning time is adjusted by the proportion of fuel and oxidizer and by the use of the cooling agent (Cichowicz and Wentzel 1983). The properties of dyes that make them suitable for use as colored smokes are: (1) rapid volatilization at 400 to 500°C, (2) minimum decomposition, (3) molecular weight not >450, and (4) purity of color and stability of the smoke condensate in air (Shidlovskiy 1964, as cited in Chin and Borer 1983).

Although sufficient toxicity data are not available to assess the health effects of DDA, many natural and synthetic anthraquinones are active in mutagenicity assays (Brown and Brown 1976, Brown 1980). Consequently, the Army Armament Research and Development Command at Aberdeen Proving Ground, Maryland, has been conducting studies to determine the feasibility of replacing DDA in the violet smoke grenade (Smith and Gerber 1981). The two violet dyes identified as replacement candidates are Disperse Red 11 and Disperse Blue 3 (Kelly J.A., USABRDL, Personal communication 1987).

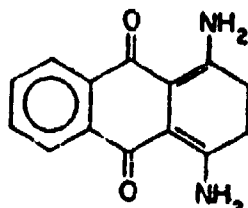
The production and use of violet-colored smoke grenades could result in environmental contamination and human exposure to DDA and its combustion products. Therefore, the objective of this report is to review the available literature concerning the environmental fate, aquatic toxicity, and mammalian toxicity of this dye in order to generate water quality criteria using the current U.S. Environmental Protection Agency (USEPA) guidelines. Current USEPA guidelines used to derive these criteria are summarized in the appendixes.

## 1.1 PHYSICAL AND CHEMICAL PROPERTIES

The physical and chemical properties of DDA are listed as follows:

CAS registry No.: 81-63-0  
Chemical name: 1,4-diamino-2,3-dihydroanthraquinone  
Synonym, trade names: 1,4-diamino-2,3-dihydro-9,10-anthracenedione  
(9 C.I.) (MEDLARS II [CHEMLINE] 1987)

Structural formula:  
(Dacre et al. 1979)



Molecular formula:  $C_{14}H_{12}N_2O_2$   
Molecular weight: 240.26 (Kitchens et al. 1978, Dacre et al. 1979)  
Physical state: Yellowish-brown powder (Rubin and Buchanan 1983)  
Melting point ( $^{\circ}C$ ): 256 (decomposition) (Dacre et al. 1979)  
Density (g/mL):  $0.35 \pm 0.10$  (Cichowicz and Wentzel 1983)  
Solubility: Insoluble in water; soluble in glacial acetic acid and hot ethanol (Dacre et al. 1979); soluble in acetone (Slaga et al. 1985); very slightly soluble in chloroform (Rubin et al. 1983)  
Explosibility index (dust): 1.0 (moderate to strong explosion) (Dorsett and Nagy 1968)  
UV absorption ( $\lambda$  max, nm): 253 (Rubin and Buchanan 1983)

## 1.2 MANUFACTURING AND ANALYTICAL TECHNIQUES

### 1.2.1 Manufacturing

According to Deiner and Polley (1972), the production of DDA starts with the reaction of alizarin, in the presence of manganous oxide, sulfuric acid, and boric acid, to form purpurin. Purpurin is then reacted with aluminum, sulfuric acid, and boric acid to form 1,4-dihydroxy-2,3-dihydroanthraquinone, which is subsequently reacted with ammonia gas under pressure to form DDA (Rys and Zollinger 1970 as cited in Kitchens et al. 1978, Deiner and Polley 1972).

The manufacturers of DDA include the following: American Cyanamid Co., Bound Brook, New Jersey; Atlantic Chemical Industry, Nutley, New Jersey; Kewanee Industrial Corporation, Louisville, Kentucky; and Toms River Chemical Corporation, Toms River, New Jersey (SRI 1977, as cited in Kitchens et al. 1978). Chem Sources-USA (1982) listed the following manufacturers as sources of DDA: Aceto Chemical Co., Carlstadt, New Jersey, and Long Island City, New York; ICN K & K Laboratories, Plainview, New York; and Pfaltz and Bauer, Inc. (Division of Aceto Chemical Co.), Stamford, Connecticut.

Table 1 shows the U.S. production levels of DDA from 1968 to 1976 and the quantity used by the military from 1965 to 1973. In 1978, the annual military usage of DDA was 3,500 lb/year, 0.7 percent of 1976 civilian production. Full mobilization usage for 1978 was estimated as 78,000 lb/year, which was 14.7 percent of 1976 civilian production or approximately 7.4 percent of 1976 civilian capacity (Kitchens et al. 1978).

Violet smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Pine Bluff, Arkansas, using the Glatt mixing process. A fluidized bed granulator combines the three operations of mixing, granulation, and drying. This technique is designed to reduce cost, improve efficiency, and provide better engineering controls for material containment, thereby reducing worker exposure to dust and the pollutant discharge of acetone (Garcia et al. 1982). The formulation of M18 violet smoke grenades is as follows: 42 percent violet smoke mixture (20 percent Disperse Red 9 and 80 percent DDA), 24 percent sodium bicarbonate, 25 percent potassium chlorate, and 9 percent sulfur (Military Specification 1970).

### 1.2.2 Analytical Techniques

The methods by which substances in violet smoke mixture and the DDA standard dye are separated, analyzed, and identified include the following: analytical and preparative scale thin-layer chromatography (TLC), visible, ultraviolet, and fluorescence spectrophotometry, capillary column

TABLE 1. U.S. PRODUCTION AND MILITARY USE LEVELS OF DDA<sup>a,b</sup>

Category	1955	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976
Production	-	-	-	754	438	651	653	952	890	834	470	531
Military <sup>c</sup>	15	46	-	-	-	21	40	33	15	-	-	-

a. Adapted from Kitchens et al. 1978.

b. Quantities reported in 10<sup>3</sup> lb; DDA - 1,4-diamino-2,3-dihydroanthraquinone.

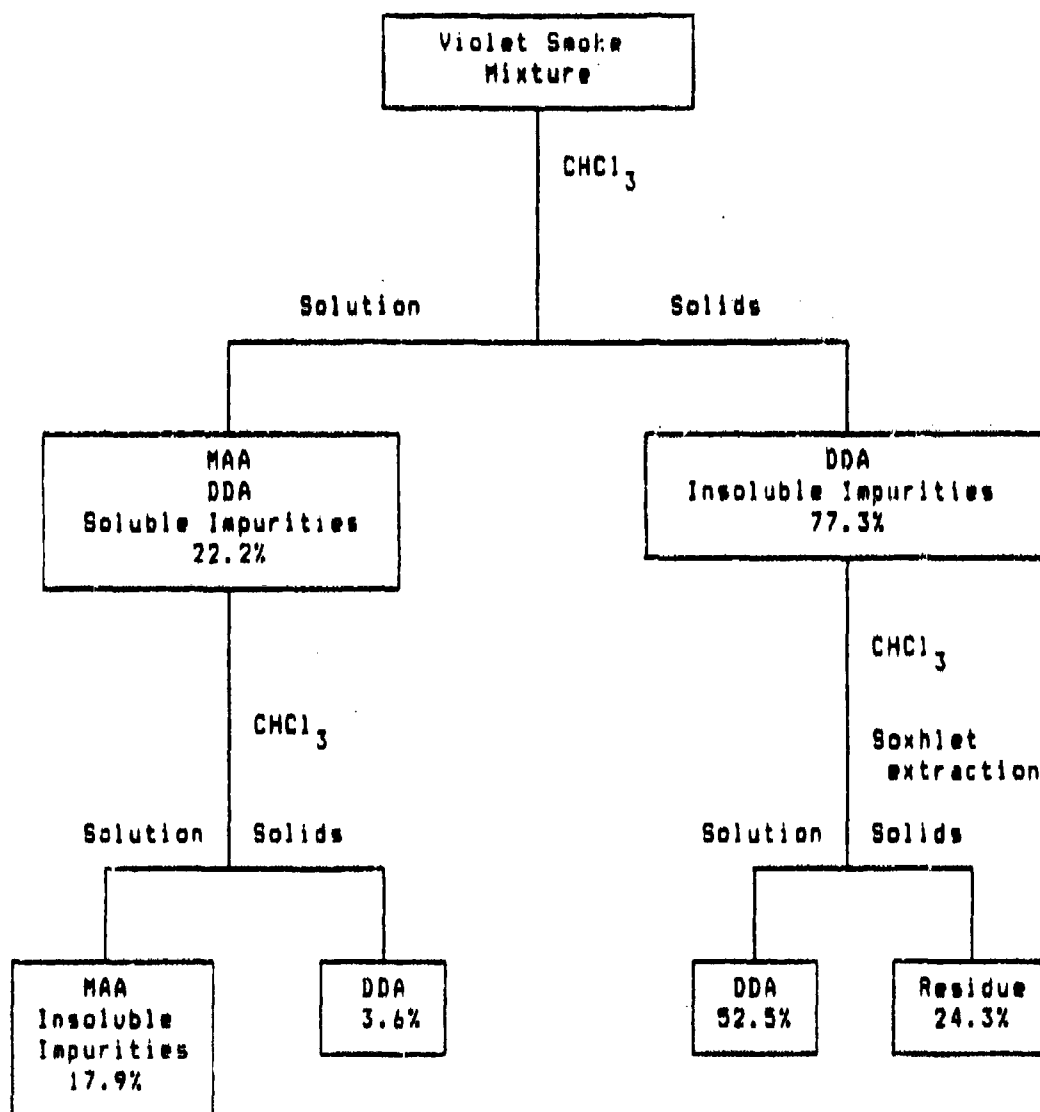
c. Quantities of DDA for military use were calculated as 80% of quantity of violet dye mixture used.

gas chromatography/mass spectrometry (GC/MS), packed column gas chromatography (GC), and  $^{13}\text{C}$  nuclear magnetic resonance (NMR). The solvent system for TLC consists of methyl ethylketone:chloroform:acetic acid (80:60:1), and the gas chromatograph is equipped with a flame ionization detector (Rubin, Buchanan, and Olerich 1982; Rubin and Buchanan 1983).

Analysis of violet smoke mixture is complicated by changes in composition of the dye mixture, which occur under various conditions. Owens and Ward (1974) observed that peaks in the visible spectrum shifted from 458 and 486  $\text{m}\mu$  before dissemination to 548 and 588  $\text{m}\mu$  after dissemination of the smoke from the grenade. The ultraviolet spectrum showed only one peak, at 248  $\text{m}\mu$ , before dissemination, but two peaks, at 244 and 394  $\text{m}\mu$ , after dissemination. In addition, Rubin, Buchanan, and Olerich (1982) and Rubin and Buchanan (1983) reported that, prior to dissemination, violet smoke mixture was composed of three major components instead of two. The third component was resolved by separating a DDA standard by TLC (Rubin and Buchanan 1983). Two bands were resolved, a broad yellow-brown band identified as DDA and a thin purple band identified as DAA. Capillary column GC/MS resolved two peaks, with molecular weights of 238 and 240, corresponding to DDA and DAA, respectively. The identity of DDA was confirmed by a  $^{13}\text{C}$  NMR spectral analysis of the standard dye. Rubin and Buchanan (1983) also observed that the yellow-brown sample changed to purple when it was warmed in the NMR probe overnight. This color change indicated that DDA was converted to DAA; the conversion was confirmed by  $^{13}\text{C}$  NMR spectral analysis. Therefore, depending on the conditions, violet smoke mixture will contain varying amounts of DAA.

The method for separating the major components in violet smoke mixture was based on chloroform solubility, because Disperse Red 9 is 70 times more soluble in chloroform than DDA (Rubin, Buchanan, and Olerich 1982; Rubin and Buchanan 1983). The separation scheme is presented in Figure 1. The components in each fraction were identified by one or more of the following methods: analytical TLC; visible, ultraviolet, or fluorescence spectrophotometry; and capillary column GC/MS. The major component in fraction 1 was Disperse Red 9, and the minor components were anthraquinone, DDA, aminonaphthalene, and aminoanthraquinone. The major component in fraction 2 was DDA with varying amounts of DAA; the minor components were Disperse Red 9, aminoanthraquinone, aminonaphthalene, and anthraquinone. The major components in fraction 3 were DDA and DAA; the only minor component identified was aminonaphthalene. The direct-probe mass spectrum showed that fraction 4 consisted of DDA (or DAA) and carbon residues (Rubin et al. 1983).

Rubin, Buchanan, and Moneyhun (1982) and Rubin et al. (1983) described methods for separating and analyzing the particulate material and vapor phase samples of combusted violet smoke. Because the particulate material was 83 percent soluble in chloroform, this fraction was separated by silica gel open-column liquid chromatography using chloroform for elution. Four bands were eluted by chloroform, and a fifth band was eluted by acetone followed by methanol. The components in each band were identified by capillary column GC/MS. Band 1 was pure Disperse Red 9 and 1-diaminoanthraquinone was the major constituent in Band 2. Band 4 was pure DAA. The vapor phase samples collected in XAD-2 and Tenax traps were extracted and analyzed by capillary-column GC/MS.



Fraction: 1

2

3

4

Figure 1. Separation Scheme for Violet Smoke Mixture Containing DDA and Disperse Red 9 (MAA) (from Rubin and Buchanan 1983).



## 2. ENVIRONMENTAL EFFECTS AND FATE

### 2.1 ABIOTIC ENVIRONMENTAL EFFECTS

No information was found in the literature concerning the abiotic effects of DDA.

### 2.2 ENVIRONMENTAL FATE

#### 2.2.1 Sources and Transport

DDA may be released into the environment during manufacture of the dye, during formulation and loading of the violet-colored smoke grenades, or during training and testing operations. Colored smoke grenades are formulated and loaded at the Pine Bluff Arsenal, Arkansas. Kitchens et al. (1978) reported that, during typical production of pyrotechnic items, approximately 1 to 2 percent of the smoke formulation or an estimated 3 to 6 lb of DDA would be discharged per month into receiving waters within the area of the arsenal. At full mobilization, 65 to 130 lb of DDA would be released per month (Kitchens et al. 1978). Combustion products resulting from detonation of the grenades can enter the aquatic environment directly as fallout, by runoff, or by leaching from soils, but the impact is usually local in nature, within 10 to 15 km downwind of the site (Cichowicz and Wentzel 1983).

Four main aquatic systems within the arsenal grounds that could receive pyrotechnic discharges drain into the Arkansas River, which fronts the arsenal for approximately six miles. Three of the aquatic systems originate on the installation. They are Triplett Creek, Yellow Creek (with associated drainages), and McGregor Reach. The fourth, Eastwood Bayou, originates off the installation. There is also an aquifer below the arsenal. The pyrotechnic complex is located just southwest of Yellow Lake. A pollution abatement facility installed in 1979 was expected to reduce the effluent discharges to these streams (Fortner et al. 1979, as cited in Kitchens et al. 1978); however, no data are available concerning current waste loading. Prior to 1979, untreated pyrotechnic wastes were discharged directly into the receiving aquatic systems that flow into the Arkansas River, producing significant contamination (Kitchens et al. 1978). Pinkham et al. (1977, as cited in Kitchens et al. 1978) reported contamination, including pyrotechnic residues and smoke mixtures, within Yellow Lake and within a munitions test area on the Arkansas River.

#### 2.2.2 Degradation and Combustion Products

Limited information was found in the literature concerning the physical, chemical, or biological degradation and/or transformation of DDA. The dye is insoluble in water; it is also readily oxidized to DAA

in air or by manganese dioxide (Rubin and Buchanan 1983, Cichowicz and Wentzel 1983).

Deiner and Polley (1972) and Deiner (1982) stated that oxidation is the means by which DDA is degraded during combustion of the violet smoke. Analysis of the combusted violet smoke using GC/MS indicated that DDA is completely converted to DAA during combustion (Rubin, Buchanan, and Moneyhun 1982, Rubin et al., 1983). Flege (1970, as cited in Dacre et al. 1979) reported that 10 percent of DAA will degrade after 15 days of aeration with domestic sewage microorganisms.

Cichowicz and Wentzel (1983) reported that DDA will undergo photodecomposition at rates dependent on surrounding environmental conditions.

### 2.3 SUMMARY

DDA may be released into the environment either during manufacturing, during formulation and loading of smoke grenades, or upon detonation of M18 colored smoke grenades during training and testing operations. The primary aquatic system receiving wastewaters from the production of violet smoke grenades at the Pine Bluff Arsenal, Arkansas, is the Arkansas River and its associated drainages. Past contamination of these systems by pyrotechnic residues has been reported as significant; however, wastewater treatment, which began in 1979, should reduce effluent discharges to acceptable levels.

DDA is readily oxidized to DAA in air and is completely converted to DAA during combustion of the violet smoke mixture. DDA is insoluble in water; however, in order to determine the fate of the dye in the environment, information concerning its transformation or transport in soil, water, and sediments must be obtained.

### 3. AQUATIC TOXICOLOGY

#### 3.1 ACUTE TOXICITY IN ANIMALS

No information was found in the literature concerning the acute toxicity of DDA in aquatic organisms.

#### 3.2 CHRONIC TOXICITY IN ANIMALS

No information was found in the literature concerning the chronic toxicity of DDA in aquatic organisms.

#### 3.3 TOXICITY IN MICROORGANISMS AND PLANTS

No information was found in the literature concerning the toxicity of DDA in microorganisms and plants.

#### 3.4 BIOACCUMULATION

No information was found in the literature concerning the bioaccumulation of DDA by aquatic organisms. However, the calculated octanol-water partition coefficient for the dye is 0.0456 (G. L. Baughman, USEPA, Personal communication 1987). The value was calculated by the substituent approach of Leo et al. (1971), based on computations used in the computer program CLOGP. Therefore, according to O'Bryan and Ross (1986), the bioaccumulation of DDA would be negligible, with an estimated bioconcentration factor of <10.

#### 3.5 OTHER DATA

Little et al. (1974) investigated the acute toxicity of selected commercial dyes in Pimephales promelas (fathead minnow) and found that pH may affect the toxicity by influencing the degree of ionization and the site of action of the dye within the organism. Consequently, if the dye is discharged with other materials that are either acid or alkaline in nature, the toxic effect may be altered.

Lagrange (1946, as cited in Dacre et al. 1979) reported that solutions of DAA, the oxidation product of DDA, killed earthworms in 15 to 30 min at concentrations of  $\leq 500$  mg/L and in <20 hr at a concentration of 50 mg/L.

#### 3.6 SUMMARY

No information was found concerning the acute and chronic toxic effects of DDA in aquatic organisms. Based on the calculated log  $k_p$ , bioaccumulation in aquatic organisms should be negligible.

#### 4. MAMMALIAN TOXICOLOGY AND HUMAN HEALTH EFFECTS

##### 4.1 PHARMACOKINETICS

No data were retrieved on the pharmacokinetics of DDA in laboratory animals or in humans.

##### 4.2 ACUTE TOXICITY

###### 4.2.1 Animal Data

Only one report was found on the acute toxic effects of DDA in laboratory animals. Seven species of animals, namely, monkeys, dogs, swine, goats, rabbits, rats, and guinea pigs, were exposed to violet smoke disseminated from a grenade (Owens and Ward 1974). The grenades contained 42 percent (by weight) violet smoke mixture, and the mixture contained 80 percent DDA and 20 percent Disperse Red 9. Owens and Ward (1974) performed a spectral analysis on the violet smoke mixture before and after dissemination from the grenades. They observed a shift in the visible and ultraviolet absorption spectra, indicating that DDA was altered during the combustion.

The exposure concentrations, maintained by sequentially firing grenades within the exposure chambers, ranged from 1,344 to 7,830 mg/m<sup>3</sup>. The exposure time ranged from 8 to 142 min, and the concentration x time (Ct) of exposure ranged from 11,626 to 858,262 mg·min/m<sup>3</sup>. The animals were observed for 30 days for mortality and for signs of toxicity (Owens and Ward 1974).

Signs of acute toxicity and time of onset are summarized in Table 2. The mortality rate was very high initially, with 33 percent of the animals dying within 24 hr; a total of 36 percent of the animals died during the experiment. The mortality rates and LC<sub>50</sub>'s are presented in Table 3. The LC<sub>50</sub> was 211,203 mg·min/m<sup>3</sup> for all species combined, 206,393 mg·min/m<sup>3</sup> for all rodents (rats and guinea pigs), and 160,013 mg·min/m<sup>3</sup> for all nonrodents (monkeys, dogs, goats, swine, and rabbits). Monkeys were the most sensitive species followed in descending order by rabbits, guinea pigs, rats, dogs, swine, and goats (Owens and Ward 1974).

Because the animals were exposed to Disperse Red 9 in addition to DDA, the toxic effects of the violet smoke mixture could not be attributed to either dye alone. Owens and Ward (1974) also exposed the same species to red smoke (Disperse Red 9 only) and observed that the LC<sub>50</sub> for all species combined was 3 times greater than that observed for violet smoke mixture, 2 times greater for all rodents, and approximately 6 times greater for all nonrodents. These data showed that violet smoke mixture was more toxic than red smoke mixture and that the toxicity of violet smoke mixture was probably due to DDA, which comprised 80 percent of the violet smoke mixture.

TABLE 2. TOXICITY AND TIME OF ONSET IN SEVEN SPECIES FOLLOWING  
INHALATION OF VIOLET SMOKE DISSEMINATED FROM THE M18 GRENADE<sup>a</sup>

Toxic Signs	Time of Onset (hr)						G.P. <sup>b</sup>
	Dog	Swine	Goat	Monkey	Rabbit	Rat	
Dyspnea	1	1	1	1	1	1	0.25
Gagging	1	-	1	1	1	1	1
Vomiting	1-24	1	-	-	-	-	-
Wheezing	1-24	-	1-48	1-48	1-24	1	1
General weakness	1-48	1	1	1-24	1-24	1	1
Ataxia	1-24	1	1	-	-	-	-
Prostration	1-48	0.5-1	1	1-48	1	1	1
Death	1-96	1-48	1-48	1-652	1-504	0.5-168	1-480

a. Adapted from Owens and Ward 1974.

b. Guinea pig.

TABLE 3. ACUTE TOXICITY IN SEVEN ANIMAL SPECIES FOLLOWING  
INHALATION OF VIOLET SMOKE DISPERSED FROM AN M18 GRENADE<sup>a, b</sup>

Species	Gt (mg·min/m <sup>3</sup> )	Conc <sub>i</sub> (mg/m <sup>3</sup> )	Exposure time (min)	Mortality (%)	LCt <sub>50</sub> <sup>c</sup> (mg·min/m <sup>3</sup> )
Monkey	437,900	7,063	62	100	39,731
	349,440	5,636	62	83	
	127,240	3,030	42	83	
	63,166	1,344	47	83	
	21,112	2,111	10	50	
	19,500	2,438	8	0	
Dog	656,882	10,595	62	100	349,950
	353,012	5,694	62	100	
	349,400	5,635	62	33	
	133,404	3,176	42	0	
	63,166	1,462	47	0	
Swine	858,262	6,131	142	100	380,753
	662,230	4,834	137	83	
	464,444	7,491	62	83	
	437,900	7,830	62	100	
	394,480	6,363	62	33	
	373,002	6,016	62	67	
	349,440	5,636	62	0	
	293,822	3,194	92	50	
	279,106	6,645	42	0	
Goat	662,230	4,834	137	100	399,831
	437,900	7,830	62	100	
	394,480	6,363	62	33	
	349,440	5,636	62	0	
Rabbit	464,444	7,491	62	100	114,756
	349,400	5,635	62	83	
	110,242	2,625	42	33	
	109,602	2,610	42	50	
	65,002	2,408	27	33	
	39,112	3,259	12	17	
	11,626	1,453	8	0	

TABLE 3. (Cont'd.)

Species	Ct (mg·min/m <sup>3</sup> )	Conc. (mg/m <sup>3</sup> )	Exposure time (min)	Mortality (%)	LCt <sub>50</sub> <sup>c</sup> (mg·min/m <sup>3</sup> )
Rat	464,444	7,491	62	100	240,130
	349,400	5,635	62	95	
	279,106	6,645	42	15	
	110,242	2,624	42	15	
	109,602	2,610	42	5	
	65,002	2,408	27	0	
	56,436	1,764	32	5	
	39,112	3,259	12	0	
Guinea pig	349,400	5,635	62	100	176,448
	279,106	6,645	42	40	
	110,242	2,624	42	35	
	109,602	2,610	42	35	
	65,002	2,408	27	15	
	56,436	1,764	32	5	
	39,112	3,259	12	0	

a. Adapted from Owens and Ward 1974.

b. Total number of animals: monkeys, dogs, goats, swine, and rabbits - 6/dose; rats and guinea pigs - 20/dose.

c. Lethal concentration x time causing 50% mortality; represents the Bliss statistical analysis of the dose-response data.

#### 4.2.2 Human Data

No data were found on the acute toxic effects of DDA in humans.

#### 4.3 SUBCHRONIC AND CHRONIC TOXICITY

No data were found on subchronic or chronic toxic effects of DDA in laboratory animals or humans.

#### 4.4 GENOTOXICITY

##### 4.4.1 Animal Data

Brown and Brown (1976) tested 90 anthraquinone derivatives, including DDA and the combustion or oxidation product, DAA, for mutagenicity in Salmonella typhimurium strains TA1535, TA100, TA1537, TA1538, TA1978, and TA98. The compounds were dissolved in 0.1 to 0.4 mL of dimethylsulfoxide

(DMSO). DDA was tested at 50, 100, and 500  $\mu$ g and DAA at 100, 500, 1,000, and 2,000  $\mu$ g, using the plate test method. Strain TA1537 gave a weak response to DDA with and without S9 metabolic activation, strain TA98 gave a marginal response with S9 metabolic activation, and the remaining strains gave negative responses. DDA was also toxic to strains TA1535, TA100, and TA98. DAA was a strong mutagen in strains TA1537, TA1538, and TA98 in the presence of S9 activation.

These results showed that, although DDA is a weak mutagen, DAA, produced by oxidation upon handling or by combustion after detonation of violet smoke grenades, is a strong mutagen.

#### 4.4.2 Human Data

No data were found on the genotoxicity of DDA in humans.

### 4.5 DEVELOPMENTAL/REPRODUCTIVE TOXICITY

No data were found on the developmental or reproductive toxicity of DDA in laboratory animals or in humans.

### 4.6 ONCOGENICITY

#### 4.6.1 Animal Data

Violet smoke mixture was tested for carcinogenicity and tumor initiating activity in the SENCAR Mouse Skin Bioassay System (Slaga et al. 1985). Violet smoke mixture, composed of 80 percent DDA and 20 percent Disperse Red 9, was dissolved in 0.2 mL of acetone and applied to the shaved backs of 40 SENCAR mice per group (20/sex). To test violet smoke as a complete carcinogen, each group was treated as follows: (1) application of 2 mg of the mixture followed 7 days later by twice weekly applications of 1 mg for 30 weeks, (2) application of 1 mg of the mixture followed 7 days later by once weekly applications of 1 mg for 30 weeks, or (3) application of 0.1 mg followed 7 days later by once weekly applications of 0.1 mg for 30 weeks. Positive controls received 2.52  $\mu$ g of 7,12-dimethylbenz(a)anthracene (DMBA) followed 7 days later by once weekly applications of the same amount. No skin papillomas or carcinomas were induced by the violet smoke mixture.

Slaga et al. (1985) also tested tumor initiating activity by applying 0.1, 1, or 2 mg of violet smoke mixture in 0.2 mL of acetone to the skin followed 7 days later by twice weekly applications of 2  $\mu$ g of 12-O-tetradecanoylphorbol-13-acetate (TPA) for 30 weeks. No carcinomas were induced. The mean papillomas/mouse were 0.05, 0.075, and 0.025 for the groups treated with 2, 1, and 0.1 mg, respectively. The mean response of the negative controls (acetone followed by TPA) was 0.025, and the response of the positive controls (DMBA followed by TPA) was 10.8.



Therefore, the response of the treated animals was not significantly different from that of the negative controls.

#### 4.6.2 Human Data

No data were found on the oncogenicity of DDA in humans.

#### 4.7 SUMMARY

Very few data are available on the toxicity of DDA. No data are available on pharmacokinetics or subchronic, chronic, developmental, and reproductive toxicity in laboratory animals. No data are available on any of the toxicity parameters in humans.

One study on the acute effects of seven animal species exposed by inhalation to violet smoke disseminated from grenades showed that monkeys are the most sensitive species, followed in descending order by rabbits, guinea pigs, rats, dogs, swine, and goats. The  $LC_{50}$  is 211,205  $\text{mg}\cdot\text{min}/\text{m}^3$  for all the species combined, 206,393  $\text{mg}\cdot\text{min}/\text{m}^3$  for all rodents (rats and guinea pigs), and 160,013  $\text{mg}\cdot\text{min}/\text{m}^3$  for all nonrodents (monkeys, dogs, goats, swine, and rabbits). Because the violet smoke mixture, composed of 80 percent DDA and 20 percent Disperse Red 9, is more toxic than red smoke, which is composed entirely of Disperse Red 9, the toxic component in violet smoke mixture is probably DDA. Moreover, because DDA is quantitatively converted to DAA during combustion, the ultimate toxic component is probably DAA.

DDA is a weak mutagen in Salmonella typhimurium, but its combustion or oxidation product, DAA, is a strong mutagen. Violet smoke mixture is noncarcinogenic in the SENCAR Mouse Skin Bioassay System.

## 5. CRITERION FORMULATION

### 5.1 EXISTING GUIDELINES AND STANDARDS

Standards for occupational exposure or exposure of the general population specifically to DDA do not exist. During the production of colored smoke grenades, workers are exposed to fine-powdered dusts. The U.S. Occupational Safety and Health Administration (USOSHA) standards (8-hr time-weighted average) for the levels of inert or nuisance dust in the occupational environment is  $15 \text{ mg/m}^3$  of total dust or  $5 \text{ mg/m}^3$  of respirable dust (USOSHA 1986). The threshold limit value for inert or nuisance dust is  $10 \text{ mg/m}^3$  of total dust or  $5 \text{ mg/m}^3$  of respirable dust (ACGIH 1986, ILO 1980). The Federal Ambient Air Quality Standard for particulate matter is  $75 \text{ } \mu\text{g/m}^3$  annual geometric mean and  $260 \text{ } \mu\text{g/m}^3$  for a maximum 24-hr concentration not to be exceeded more than once per year (USEPA 1981, as cited in Cichowicz and Wentzel 1983).

The Surgeon General of the Army has established interim guidelines for the disposal of colored smokes. There should be no open burning, and personnel should not be exposed to dye components at levels above  $0.2 \text{ mg/m}^3$  (8-hr time-weighted average) (Cichowicz and Wentzel 1983).

### 5.2 OCCUPATIONAL EXPOSURE

Occupational exposure standards specifically for DDA do not exist. Manufacturing personnel are exposed to fine-powdered dusts through inhalation, skin, and eye contact. According to Garcia et al. (1982), the levels of dust in the colored smoke grenade production facility at the Pine Bluff Arsenal, Pine Bluff, Arkansas, exceeded the limits established by USOSHA. During training and testing operations, Army personnel are exposed to pyrolysis reaction products formed from combustion of colored smoke grenades and, upon dissemination, to dye vapors as condensate in the smoke cloud (Tatyrek 1965).

### 5.3 PREVIOUSLY CALCULATED CRITERIA

No aquatic or human health criteria have been previously calculated for DDA.

### 5.4 AQUATIC CRITERIA

A brief description of the methodology proposed by the USEPA for the estimation of water quality criteria for the protection of aquatic life and its uses is presented in Appendix A. The aquatic criteria consist of two values, a Criterion Maximum Concentration (CMC) and a Criterion Continuous Concentration (CCC) (Stephan et al. 1985). The CMC is equal to one-half the Final Acute Value (FAV), whereas the CCC is equal to the

lowest of the Final Chronic Value, the Final Plant Value, or the Final Residue Value.

No data are available in the literature concerning the toxicity of DDA in aquatic organisms; consequently, neither a CMC nor a CCC can be calculated.

## 5.5 HUMAN HEALTH CRITERIA

No data were retrieved on the carcinogenicity of DDA in humans. Violet smoke mixture containing DDA and Disperse Red 9 was not carcinogenic in the SENCAR Mouse Skin Bioassay System (Slaga et al. 1985). Therefore, a criterion based on carcinogenicity (nonthreshold toxicity data) cannot be calculated. No data were retrieved on the subchronic or chronic toxicity in humans or in laboratory animals. Therefore, a criterion based on chronic toxicity (threshold toxicity data) cannot be calculated. Numerous data gaps will have to be filled before a criterion can be established. These conclusions were based on the USEPA guidelines for deriving a water quality criterion for the protection of human health (USEPA 1980) summarized in Appendix B.

## 5.6 RESEARCH RECOMMENDATIONS

Since DDA will be replaced in violet smoke grenades by a new candidate dye, toxicity tests will only be necessary if large quantities are on hand that require disposal. To meet the requirements established by the USEPA for deriving water quality criteria, the following research studies are recommended to fill gaps in the existing data.

1. Observations that DDA is readily converted to DAA under various conditions lead to uncertainty concerning its presence in environmental media. Prior to conducting aquatic and mammalian toxicity tests, environmental (air, soil, water, and sediment) samples should be taken to determine the occurrence and fate of DDA and its oxidation product DAA, in the environment and in the workplace. If DDA does not remain stable in these media, then it will be necessary to decide which compound should be tested.
2. To calculate a FAV, perform acute toxicity tests following USEPA procedures and the American Society for Testing and Materials (ASTM 1980) methods for at least eight different families of aquatic organisms: (a) member of family *Almonidae* in class Osteichthyes; (b) member of second family in class Osteichthyes, preferably an important warmwater species; (c) member of a third family in phylum Chordata; (d) planktonic crustacean; (e) benthic crustacean; (f) member of class Insecta; (g) member in phylum other than Arthropoda or Chordata; and (h) member of family in any order of class Insecta or any phylum not represented.

3. Conduct chronic flow-through tests, using measured concentrations for an invertebrate species, a fish species, and a sensitive freshwater species in order to calculate a Final Chronic Value.
4. Conduct acute flow-through tests, using measured concentrations for the three aquatic species for which chronic tests are being performed in order to calculate acute-chronic ratios.
5. Conduct a conclusive toxicity test with an alga or aquatic vascular plant, using measured concentrations and a biologically important end point in order to calculate a Final Plant Value.
6. Conduct a definitive steady-state or 28-day bioaccumulation study. Determine a maximum permissible tissue concentration by conducting a chronic wildlife feeding study or a long-term wildlife field study. These data will provide information for calculating a Final Residue Value.
7. Conduct the following tests in rats or mice, according to USEPA Toxic Substances Control Act Test Guidelines (USEPA 1985): acute oral toxicity tests, acute dermal toxicity tests, oral subchronic/chronic toxicity tests, (which would also include a carcinogenicity end point), teratogenicity and reproductive toxicity tests, in vitro genotoxicity tests in mammalian cells, and in vivo genotoxicity tests (dominant lethal tests in mice or rats and/or chromosome aberration in mouse bone marrow cells).

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## 7. GLOSSARY

ACGIH	American Conference of Governmental Industrial Hygienists
ASTM	American Society for Testing and Materials
CCC	Criterion Continuous Concentration
CMC	Criterion Maximum Concentration
Ct	Concentration x time
DAA	1,4-Diaminoanthraquinone
DDA	1,4-Diamino-2,3-dihydroanthraquinone
DMBA	7,12-Dimethylbenz(a)anthracene
DMSO	Dimethylsulfoxide
FAV	Final Acute Value
GC	Gas Chromatography
GC/MS	Gas Chromatography/Mass Spectrometry
ILO	International Labor Office
LCt <sub>50</sub>	Lethal Concentration x time causing 50% mortality
MAA	Disperse Red 9 (1-methylaminoanthraquinone)
NMR	Nuclear Magnetic Resonance
TLC	Thin-Layer Chromatography
TPA	12-O-Tetradecanoylphorbol-13-acetate
USEPA	United States Environmental Protection Agency
USOSHA	United States Occupational Safety and Health Administration

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## APPENDIX A

### SUMMARY OF USEPA METHODOLOGY FOR DERIVING NUMERICAL WATER QUALITY CRITERIA FOR THE PROTECTION OF AQUATIC ORGANISMS AND THEIR USES

The following summary is a condensed version of the 1985 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect aquatic life with emphasis on the specific regulatory needs of the U.S. Army (e.g., discussion of saltwater aspects of the criteria calculation are not included). The guidelines are the most recent document outlining the required procedures and were written by the following researchers from the USEPA's regional research laboratories: C.E. Stephan, D.I. Mount, D.J. Hansen, J.H. Gentile, G.A. Chapman, and W.A. Brungs. For greater detail on individual points consult Stephan et al. (1985).

#### 1. INTRODUCTION

The Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses describe an objective, internally consistent, and appropriate way of estimating national criteria. Because aquatic life can tolerate some stress and occasional adverse effects, protection of all species at all times was not deemed necessary. If acceptable data are available for a large number of appropriate taxa from a variety of taxonomic and functional groups, a reasonable level of protection should be provided if all except a small fraction are protected, unless a commercially, recreationally, or socially important species is very sensitive. The small fraction is set at 0.05, because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is appropriate.

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term and short-term effects on (1) commercially, recreationally, and socially important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. These national guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations.

Numerical aquatic life criteria derived using these national guidelines are expressed as two numbers, so that the criteria can more accurately reflect toxicological and practical realities. The combination of a maximum concentration and a continuous concentration is designed to provide adequate protection of aquatic life and its uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by

aquatic organisms without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these guidelines should be useful for developing water quality standards, mixing zone standards, and effluent standards. Development of such standards may have to consider additional factors such as social, legal, economic, and additional biological data. It may be desirable to derive site-specific criteria from these national criteria to reflect local conditions (USEPA 1982). The two factors that may cause the most difference between the national and site-specific criteria are the species that will be exposed and the characteristics of the water.

Criteria should provide reasonable and adequate protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a criterion be the best estimate obtainable using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Thus, these guidelines require that certain data be available if a criterion is to be derived. If all the required data are not available, usually a criterion should not be derived; however, availability of all required data does not ensure that a criterion can be derived. The amount of guidance in these national guidelines is significant, but much of it is necessarily qualitative rather than quantitative; much judgement will be required to derive a water quality criterion for aquatic life. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these guidelines and should be consistent with the spirit of these guidelines - which is to make best use of all available data to derive the most appropriate criterion.

## 2. DEFINITION OF MATERIAL OF CONCERN

1. Each separate chemical that does not ionize significantly in most natural bodies of water should be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.
2. For chemicals that do ionize significantly, all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.
3. Definition of the material should include an operational analytical component. It is also necessary to reference or describe analytical methods that the term is intended to denote. Primary requirements of the operational analytical component is that it be appropriate for use on samples of receiving water, that it be compatible with toxicity and

bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection of aquatic life and its uses.

**NOTE:** Analytical chemistry of the material may have to be considered when defining the material or when judging acceptability of some toxicity tests, but a criterion should not be based on sensitivity of an analytical method. When aquatic organisms are more sensitive than analytical techniques, the proper solution is to develop better analytical methods, not to underprotect aquatic life.

### 3. COLLECTION OF DATA

1. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants; (b) FDA action levels (FDA Guidelines Manual); and (c) chronic feeding studies and long-term field studies with wildlife that regularly consume aquatic organisms.
2. All data used should be available in typed, dated, and signed hardcopy with enough supporting information to indicate that acceptable test procedures were used and the results should be reliable.
3. Questionable data, whether published or not, should not be used.
4. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the test material should not be used.
5. For some highly volatile, hydrolyzable, or degradable materials it may be appropriate to only use results of flow-through tests in which concentrations of test material in test solutions were measured using acceptable analytical methods.
6. Do not use data obtained using brine shrimp, species that do not have reproducing wild populations in North America, or organisms that were previously exposed to significant concentrations of the test material or other contaminants.

### 4. REQUIRED DATA

1. Results of acceptable acute tests (see Section 5) with freshwater animals in at least eight different families such that all of the following are included:
  - a. the family Salmonidae in the class Osteichthyes;

- b. a second family (preferably an important warmwater species) in the class Osteichthyes (e.g., bluegill, fathead minnow, or channel catfish);
  - c. a third family in the phylum Chordata (e.g., fish or amphibian);
  - d. a planktonic crustacean (e.g., cladoceran or copepod);
  - e. a benthic crustacean (e.g., ostracod, isopod, or amphipod);
  - f. an insect (e.g., mayfly, midge, stonefly);
  - g. a family in a phylum other than Arthropoda or Chordata (e.g., Annelida or Mollusca); and
  - h. a family in any order of insect or any phylum not represented.
2. Acute-chronic ratios (see Section 7) for species of aquatic animals in at least three different families provided that of the three species at least (a) one is a fish, (b) one is an invertebrate, and (c) one is a sensitive freshwater species.
  3. Results of at least one acceptable test with a freshwater alga or a chronic test with a freshwater vascular plant (see Section 9). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should be available.
  4. At least one acceptable bioconcentration factor determined with an appropriate aquatic species, if a maximum permissible tissue concentration is available (see Section 10).

If all required data are available, a numerical criterion can usually be derived, except in special cases. For example, if a criterion is to be related to a water quality characteristic (see Sections 6 and 8), more data will be necessary. Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if acute and chronic data are not available, it may be possible to derive a criterion if the data clearly indicate that the Final Residue Value would be much lower than either the Final Chronic Value or the Final Plant Value. Confidence in a criterion usually increases as the amount of data increases. Thus, additional data are usually desirable.

## 5. FINAL ACUTE VALUE

1. The Final Acute Value (FAV) is an estimate of the concentration of material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acute tests have

been conducted on the material. However, in some cases, if the Species Mean Acute Value (SMAV) of an important species is lower than the calculated FAV, then that SMAV replaces the FAV to protect that important species.

2. Acute toxicity tests should have been conducted using acceptable procedures (e.g., ASTM Standard E 724 or 729).
3. Generally, results of acute tests in which food was added to the test solution should not be used, unless data indicate that food did not affect test results.
4. Results of acute tests conducted in unusual dilution water, e.g., dilution water containing high levels of total organic carbon or particulate matter (higher than 5 mg/L), should not be used, unless a relationship is developed between toxicity and organic carbon or unless data show that organic carbon or particulate matter, etc. do not affect toxicity.
5. Acute values should be based on endpoints which reflect the total adverse impact of the test material on the organisms used in the tests. Therefore, only the following kinds of data on acute toxicity to freshwater aquatic animals should be used:
  - a. Tests with daphnids and other cladocerans should be started with organisms < 24 hr old, and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC<sub>50</sub> based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC<sub>50</sub> is not available from a test, the 48-hr LC<sub>50</sub> should be used in place of the desired 48-hr EC<sub>50</sub>. An EC<sub>50</sub> or LC<sub>50</sub> of longer than 48 hr can be used provided animals were not fed and control animals were acceptable at the end of the test.
  - b. The result of tests with all other aquatic animal species should be the 96-hr EC<sub>50</sub> value based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC<sub>50</sub> value is not available from a test, the 96-hr LC<sub>50</sub> should be used in place of the desired EC<sub>50</sub>.
  - c. Tests with single-cell organisms are not considered acute tests, even if the duration was  $\leq$  96 hr.
  - d. If the tests were conducted properly, acute values reported as greater than values and those acute values which are above solubility of the test material are acceptable.
6. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic (e.g., total organic carbon) for freshwater species, a Final Acute Equation should be derived based on that characteristic.

7. If the data indicate that one or more life stages are at least a factor of 2 times more resistant than one or more other life stages of the same species, the data for the more resistant life stages should not be used in the calculation of the SMAV, because a species can only be considered protected from acute toxicity if all life stages are protected.
8. Consider the agreement of the data within and between species. Questionable results in comparison with other acute and chronic data for the species and other species in the same genus probably should not be used.
9. For each species for which at least one acute value is available, the SMAV should be calculated as the geometric mean of all flow-through test results in which the concentrations of test material were measured. For a species for which no such result is available, calculate the geometric mean of all available acute values, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial total concentrations of test material.

NOTE: Data reported by original investigators should not be rounded off, and at least four significant digits should be retained in intermediate calculations.

10. For each genus for which one or more SMAV is available, calculate the Genus Mean Acute Value (GMAV) as the geometric mean of the SMAVs.
11. Order the GMAVs from high to low, and assign ranks (R) to the GMAVs from "1" for the lowest to "N" for the highest. If two or more GMAVs are identical, arbitrarily assign them successive ranks.
12. Calculate the cumulative probability (P) for each GMAV as  $R/(N+1)$ .
13. Select the four GMAVs which have cumulative probabilities closest to 0.05 (If there are <59 GMAVs, these will always be the four lowest GMAVs).
14. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\sum((\ln \text{GMAV})^2) - ((\sum(\ln \text{GMAV}))^2/4)}{\sum(P) - ((\sum(\sqrt{P}))^2/4)}$$

$$L = (\sum(\ln \text{GMAV}) - S(\sum(\sqrt{P}))) / 4$$

$$A = S(\sqrt{0.05}) + L$$

$$\text{FAV} = e^A$$



15. If for an important species, such as a recreationally or commercially important species, the geometric mean of acute values from flow-through tests in which concentrations of test material were measured is lower than the FAV, then that geometric mean should be used as the FAV.
16. Go to Section 7.

## 6. FINAL ACUTE EQUATION

1. When enough data show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
2. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of acute toxicity values on values of the water quality characteristic.
3. Decide whether the data for each species is useful, considering the range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used.
4. Individually for each species calculate the geometric mean of the acute values and then divide each of the acute values for a species by the mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.
5. Similarly normalize the values of the water quality characteristic for each species individually.
6. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 2. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
7. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope (V) and its 95 percent confidence limits. If all the normalized data are

actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

8. For each species calculate the geometric mean (W) of the acute toxicity values and the geometric mean (X) of the related values of the water quality characteristic (calculated in 4. and 5. above).
9. For each species calculate the logarithmic intercept (Y) of the SMAV at a selected value (Z) of the water quality characteristic using the equation:  $Y = \ln W - V(\ln X - \ln Z)$ .
10. For each species calculate the SMAV using:  $SMAV = e^Y$ .
11. Obtain the FAV at Z by using the procedure described in Section 5 (Nos. 10-14).
12. If the SMAV for an important species is lower than the FAV at Z, then that SMAV should be used as the FAV at Z.
13. The Final Acute Equation is written as:

$$FAV = e^{(V[\ln(\text{water quality characteristic})] + \ln A - V[\ln Z])}$$

where V = pooled acute slope and A = FAV at Z. Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

## 7. FINAL CHRONIC VALUE

1. Depending on available data, the Final Chronic Value (FCV) might be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio.

NOTE: Acute-chronic ratios and application factors are ways of relating acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it should usually be greater than one; this should avoid confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

2. Chronic values should be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which concentrations of test material were properly measured at appropriate times during testing.
3. Results of chronic tests in which survival, growth, or reproduction in controls was unacceptably low should not be used. Limits of acceptability will depend on the species.

4. Results of chronic tests conducted in unusual dilution water should not be used, unless a relationship is developed between toxicity and the unusual characteristic or unless data show the characteristic does not affect toxicity.
5. Chronic values should be based on endpoints and exposure durations appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should be used:
  - a. Life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Tests with fish should begin with embryos or newly hatched young < 48 hr old, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young < 24 hr old and last for not < 21 days. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female.
  - b. Partial life-cycle toxicity tests consisting of exposures of two or more groups of a species to a different concentration of test material throughout a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. Exposure to the test material should begin with juveniles at least 2 months prior to active gonadal development, continue through maturation and reproduction, and should end not < 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.
  - c. Early life-stage toxicity tests consisting of 28- to 32-day (60 days posthatch for salmonids) exposures of early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained on growth and survival.

**NOTE:** Results of an early life-stage test are used as predictors of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test should not be used, because results of such tests may be poor estimates of results of a comparable life-cycle or partial life-cycle test.

6. A chronic value may be obtained by calculating the geometric mean of lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis. A lower chronic limit is the highest tested concentration (a) in an acceptable chronic test, (b) which did not cause an unacceptable amount of an adverse effect on any specified biological measurements, and (c) below which no tested concentration caused such an unacceptable effect. An upper chronic limit is the lowest tested concentration (a) in an acceptable chronic test, (b) which did cause an unacceptable amount of an adverse effect on one or more of specified biological measurements, and (c) above which all tested concentrations caused such an effect.
7. If chronic toxicity of the material to aquatic animals appears to be related to a water quality characteristic, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section 8.
8. If chronic values are available for species in eight families as described in Section 4 (No. 1), a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values for the species, and appropriate Genus Mean Chronic Values should be calculated. The FCV should then be obtained using procedures described in Section 5 (Nos. 10-14). Then go to Section 7 (No. 13).
9. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. Acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water may be used, or acute tests conducted in the same dilution water but a different laboratory may be used. If such acute tests are not available, an acute-chronic ratio should not be calculated.
10. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios for that species.
11. For some materials the acute-chronic ratio is about the same for all species, but for other materials the ratio increases or decreases as the SMAV increases. Thus, the Final Acute-Chronic Ratio can be obtained in three ways, depending on the data.
  - a. If the species mean acute-chronic ratio increases or decreases as the SMAV increases, the final Acute-Chronic Ratio should be

calculated as the geometric mean of all species whose SMAVs are close to the FAV.

- b. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all species mean acute-chronic ratios for both freshwater and salt-water species.
- c. If the most appropriate species mean acute-chronic ratios are  $< 2.0$ , and especially if they are  $< 1.0$ , acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be set at 2.0 so that the FCV is equal to the Criterion Maximum Concentration.

If the acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and an FCV probably cannot be calculated.

12. Calculate the FCV by dividing the FAV by the Final Acute-Chronic Ratio.
13. If the SMAV of an important species is lower than the calculated FCV, then that SMCV should be used as the FCV.
14. Go to Section 9.

#### 8. FINAL CHRONIC EQUATION

1. A Final Chronic Equation can be derived in two ways. The procedure described in this section will result in the chronic slope being the same as the acute slope.
  - a. If acute-chronic ratios for enough species at enough values of the water quality characteristics indicate that the acute-chronic ratio is probably the same for all species and independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the species mean acute-chronic ratios.
  - b. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see Section 6, No. 13) by the Final Acute-Chronic Ratio.
  - c. Use V - pooled acute slope (see Section 6, No. 13) as L - pooled chronic slope.
  - d. Go to Section 8, No. 2, item m.

2. The procedure described in this section will usually result in the chronic slope being different from the acute slope.
  - a. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be considered as described below or using analysis of covariance (Dixon and Brown 1979, Neter and Wasserman 1974). If two or more factors affect toxicity, multiple regression analyses should be used.
  - b. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of chronic toxicity values on values of the water quality characteristic.
  - c. Decide whether data for each species are useful, taking into account range and number of tested values of the water quality characteristic and degree of agreement within and between species. In addition, questionable results, in comparison with other acute and chronic data for the species and other species in the same genus, probably should not be used. If a useful chronic slope is not available for at least one species or if the slopes are too dissimilar or if data are inadequate to define the relationship between chronic toxicity and water quality characteristic, return to Section 7 (No. 5), using results of tests conducted under conditions and in water similar to those commonly used for toxicity tests with the species.
  - d. For each species calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species and for any combination of species is 1.0.
  - e. Similarly normalize the values of the water quality characteristic for each species individually.
  - f. Individually for each species perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in 1. above. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.
  - g. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope (L) and its 95 percent confidence limits. If all the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

- h. For each species calculate the geometric mean (M) of toxicity values and the geometric mean (P) of related values of the water quality characteristic.
- i. For each species calculate the logarithm (Q) of the SMCVs at a selected value (Z) of the water quality characteristic using the equation:  $Q = \ln M - L(\ln P - \ln Z)$ .
- j. For each species calculate a SMCV at Z as the antilog of Q (SMCV =  $e^Q$ ).
- k. Obtain the FCV at Z by using the procedure described in Section 5 (Nos. 10-14).
- l. If the SMCV at Z of an important species is lower than the calculated FCV at Z, then that SMCV should be used as the FCV at Z.
- m. The Final Chronic Equation is written as:

$$FCV = e^{(L[\ln(\text{water quality characteristic})] + \ln S - L[\ln Z])}$$

where L = mean chronic slope and S = FCV at Z.

#### 9. FINAL PLANT VALUE

1. Appropriate measures of toxicity of the material to aquatic plants are used to compare relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting results of toxicity tests with plants are not well developed, results of such tests usually indicate that criteria which adequately protect aquatic animals and their uses also protect aquatic plants and their uses.
2. A plant value is the result of any test conducted with an alga or an aquatic vascular plant.
3. Obtain the Final Plant Value by selecting the lowest result obtained in a test on an important aquatic plant species in which concentrations of test material were measured and the endpoint is biologically important.

#### 10. FINAL RESIDUE VALUE

1. The Final Residue Value (FRV) is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from exceeding applicable FDA action levels and (b) protect wildlife, including fish and birds, that consume aquatic organisms from demonstrated unacceptable effects. The FRV is the lowest of residue values that are obtained by dividing maximum permissible tissue

concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level (FDA administrative guidelines) for fish oil or for the edible portion of fish or shellfish or (b) a maximum acceptable dietary intake (ADI) based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section 11., because a Final Residue Value cannot be derived.

2. Bioconcentration Factors (BCFs) and Bioaccumulation Factors (BAFs) are the quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution to which the organism has been exposed. A BCF is intended to account only for net uptake directly from water, and thus almost has to be measured in a laboratory test. A BAF is intended to account for net uptake from both food and water in a real-world situation, and almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey. Because so few acceptable BAFs are available, only BCFs will be discussed further, but an acceptable BAF can be used in place of a BCF.
3. If a maximum permissible tissue concentration is available for a substance (e.g., parent material or parent material plus metabolite), the tissue concentration used in BCF calculations should be for the same substance. Otherwise the tissue concentration used in the BCF calculation should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.
  - a. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of test material in tissue and in the test solution, and exposure continued at least until either apparent steady-state (BCF does not change significantly over a period of time, such as two days or 16 percent of exposure duration, whichever is longer) or 28 days was reached. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached; (b) highest BCF obtained, if apparent steady-state was not reached; and (c) projected steady-state BCF, if calculated.
  - b. Whenever a BCF is determined for a lipophilic material, percentage of lipids should also be determined in the tissue(s) for which the BCF is calculated.
  - c. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to that obtained with unaffected individuals at lower concentrations that did cause effects.



- d. Because maximum permissible tissue concentrations are rarely based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight by 0.1 for plankton and by 0.2 for species of fishes and invertebrates.
  - e. If more than one acceptable BCF is available for a species, the geometric mean of values should be used, unless the BCFs are from different exposure durations, in which case the BCF for the longest exposure should be used.
4. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:
- a. For each available maximum ADI derived from a feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of tested wildlife species.
  - b. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.
5. For lipophilic materials, it may be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percentage of lipids from one tissue to another and from one species to another (Hamelink et al. 1971, Lundsford and Blem 1982, Schnoor 1982), extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percentage of lipids.
- a. For each BCF for which percentage of lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by percentage of lipids. This adjustment makes all the measured BCFs comparable regardless of species or tissue.
  - b. Calculate the geometric mean normalized BCF.
  - c. Calculate all possible residue values by dividing available maximum permissible tissue concentrations by the mean normalized BCF and by the percentage of lipids values appropriate to the maximum permissible tissue concentration.
    - For an FDA action level for fish oil, the appropriate percentage of lipids value is 100.

- For an FDA action level for fish, the appropriate percentage of lipids value is 11 for freshwater criteria, based on the highest levels for important consumed species (Sidwell 1981).
  - For a maximum ADI derived from a chronic feeding study or long-term field study with wildlife, the appropriate percentage of lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.
6. The FRV is obtained by selecting the lowest of available residue values.

## 11. OTHER DATA

Pertinent information that could not be used in earlier sections may be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other biologically important adverse effect. Especially important are data for species for which no other data are available.

## 12. CRITERION

1. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.
2. The Criterion Maximum Concentration (CMC) is equal to one-half of the FAV.
3. The Criterion Continuous Concentration (CCC) is equal to the lowest of the FCV, the Final Plant Value, and the FRV unless other data show a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the FRV by selecting the value or concentration that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section 11) show that a lower value should be used.
4. Round both the CCC and CMC to two significant figures
5. The criterion is stated as: The procedures described in the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses indicate that (except possibly where a locally important species is very sensitive) (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

Where,

- (1) - insert freshwater or saltwater,
- (2) - insert name of material,
- (3) - insert the Criterion Continuous Concentration, and
- (4) - insert the Criterion Maximum Concentration.

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## APPENDIX B

### SUMMARY OF USEPA METHODOLOGY FOR DETERMINING WATER QUALITY CRITERIA FOR THE PROTECTION OF HUMAN HEALTH

The following summary is a condensed version of the 1980 final U.S. Environmental Protection Agency (USEPA) guidelines for calculating water quality criteria to protect human health and is slanted towards the specific regulatory needs of the U.S. Army. The guidelines are the most recent document outlining the required procedures and were published in the Federal Register (USEPA 1980). For greater detail on individual points consult that reference.

#### 1. INTRODUCTION

The EPA's water quality criteria for the protection of human health are based on one or more of the following properties of a chemical pollutant:

(a) Carcinogenicity, (b) Toxicity, and (c) Organoleptic (taste and odor) effects.

The meanings and practical uses of the criteria values are distinctly different depending on the properties on which they are based. Criteria based solely on organoleptic effects do not necessarily represent approximations of acceptable risk levels for human health. In all other cases the values represent estimates that would prevent adverse health effects or, for suspect and proven carcinogens, estimations of the increased cancer risk associated with incremental changes in the ambient water concentration of the substance. Social and economic costs and benefits are not considered in determining water quality criteria. In establishing water quality standards, the choice of the criterion to be used depends on the designated water use. In the case of a multiple-use water body, the criterion protecting the most sensitive use is applied.

#### 2. DATA NEEDED FOR HUMAN HEALTH CRITERIA

Criteria documentation requires information on: (1) exposure levels, (2) pharmacokinetics, and (3) range of toxic effects of a given water pollutant.

##### 2.1 EXPOSURE DATA

For an accurate assessment of total exposure to a chemical, consideration must be given to all possible exposure routes including ingestion of contaminated water and edible aquatic and nonaquatic organisms, as well as exposure through inhalation and dermal contact. For water quality criteria the most important exposure routes to be considered are ingestion of water and consumption of fish and shellfish. Generally,

exposure through inhalation, dermal contact, and non-aquatic diet is either unknown or so low as to be insignificant; however, when such data are available, they must be included in the criteria evaluation.

The EPA guidelines for developing water quality criteria are based on the following assumptions, which are designed to be protective of a healthy adult male who is subject to average exposure conditions:

1. The exposed individual is a 70-kg male person (International Commission on Radiological Protection 1977).
2. The average daily consumption of freshwater and estuarine fish and shellfish products is equal to 6.5 grams.
3. The average daily ingestion of water is equal to 2 liters (Drinking Water and Health, National Research Council 1977).

Because fish and shellfish consumption is an important exposure factor, information on bioconcentration of the pollutant in edible portions of ingested species is necessary to calculate the overall exposure level. The bioconcentration factor (BCF) is equal to the quotient of the concentration of a substance in all or part of an organism divided by the concentration in ambient water to which the organism has been exposed. The BCF is a function of lipid solubility of the substance and relative amount of lipids in edible portions of fish or shellfish. To determine the weighted average BCF, three different procedures can be used depending upon lipid solubility and availability of bioconcentration data:

1. For lipid soluble compounds, the average BCF is calculated from the weighted average percent lipids in ingested fish and shellfish in the average American diet. The latter factor has been estimated to be 3 percent (Stephan 1980, as cited in USEPA 1980). Because steady-state BCFs for lipid soluble compounds are proportional to percent lipids, the BCF for the average American diet can be calculated as follows:

$$BCF_{avg} = BCF_{sp} \times \frac{3.0\%}{PL_{sp}}$$

where  $BCF_{sp}$  is the bioconcentration factor for an aquatic species and  $PL_{sp}$  is the percent lipids in the edible portions of that species.

2. Where an appropriate bioconcentration factor is not available, the BCF can be estimated from the octanol/water partition coefficient (P) of a substance as follows:

$$\log BCF = (0.85 \log P) - 0.70$$

for aquatic organisms containing about 7.6 percent lipids (Veith et al. 1980, as cited in USEPA 1980). An adjustment for percent lipids in the average diet (3 percent versus 7.6 percent) is made to derive the weighted average bioconcentration factor.

3. For nonlipid-soluble compounds, the available BCFs for edible portions of consumed freshwater and estuarine fish and shellfish are weighted according to consumption factors to determine the weighted BCF representative of the average diet.

## 2.2 PHARMACOKINETIC DATA

Pharmacokinetic data, encompassing information on absorption, distribution, metabolism, and excretion, are needed for determining the biochemical fate of a substance in human and animal systems. Information on absorption and excretion in animals, together with a knowledge of ambient concentrations in water, food, and air, are useful in estimating body burdens in humans. Pharmacokinetic data are also essential for estimating equivalent oral doses based on data from inhalation or other routes of exposure.

## 2.3 BIOLOGICAL EFFECTS DATA

Effects data which are evaluated for water quality criteria include acute, subchronic, and chronic toxicity; synergistic and antagonistic effects; and genotoxicity, teratogenicity, and carcinogenicity. The data are derived primarily from animal studies, but clinical case histories and epidemiological studies may also provide useful information. According to the EPA (USEPA 1980), several factors inherent in human epidemiological studies often preclude their use in generating water quality criteria (see NAS 1977). However, epidemiological data can be useful in testing the validity of animal-to-man extrapolations.

From assessment of all the available data, a biological endpoint, i.e., carcinogenicity, toxicity, or organoleptic effects, is selected for criteria formulation.

## 3. HUMAN HEALTH CRITERIA FOR CARCINOGENIC SUBSTANCES

If sufficient data exist to conclude that a specific substance is a potential human carcinogen (carcinogenic in animal studies, with supportive genotoxicity data, and possibly also supportive epidemiological data) then the position of the EPA is that the water quality criterion for that substance (recommended ambient water concentration for maximum protection of human health) is zero. This is because the EPA believes that no method exists for establishing a threshold level for carcinogenic effects, and, consequently, there is no scientific basis for establishing a "safe" level. To better define the carcinogenic risk associated with a

particular water pollutant, the EPA has developed a methodology for determining ambient water concentrations of the substance which would correspond to incremental lifetime cancer risks of  $10^{-7}$  to  $10^{-5}$  (one additional case of cancer in populations ranging from ten million to 100,000, respectively). These risk estimates, however, do not represent an EPA judgment as to an "acceptable" risk level.

### 3.1. METHODOLOGY FOR DETERMINING CARCINOGENICITY (NONTRESHOLD) CRITERIA

The ambient water concentration of a substance corresponding to a specific lifetime carcinogenic risk can be calculated as follows:

$$C = \frac{70 \times PR}{q_1^* (2 + 0.0065 BCF)}$$

where

- C = ambient water concentration;
- PR = the probable risk (e.g.,  $10^{-5}$ ; equivalent to one case in 100,000);
- BCF = the bioconcentration factor; and
- $q_1^*$  = a coefficient, the cancer potency index (defined below) (USEPA 1980).

By rearranging the terms in this equation, it can be seen that the ambient water concentration is one of several factors which define the overall exposure level:

$$PR = \frac{q_1^* \times C (2 + 0.0065 BCF)}{70}$$

or

$$PR = \frac{q_1^* \times 2C + (0.0065 BCF \times C)}{70}$$

where

2C is the daily exposure resulting from drinking 2 liters of water per day and  $(0.0065 BCF \times C)$  is the average daily exposure resulting from the consumption of 6.5 mg of fish and shellfish per day. Because the exposure is calculated for a 70-kg man, it is normalized to a per



kilogram basis by the factor of 1/70. In this particular case, exposure resulting from inhalation, dermal contact, and nonaquatic diet is considered to be negligible.

In simplified terms the equation can be rewritten

$$PR = q_1^* X,$$

where X is the total average daily exposure in mg/kg/day or

$$q_1^* = \frac{PR}{X},$$

showing that the coefficient  $q_1^*$  is the ratio of risk to dose, an indication of the carcinogenic potency of the compound.

The USEPA guidelines state that for the purpose of developing water quality criteria, the assumption is made that at low dose levels there is a linear relationship between dose and risk (at high doses, however, there may be a rapid increase in risk with dose resulting in a sharply curved dose/response curve). At low doses then, the ratio of risk to dose does not change appreciably and  $q_1^*$  is a constant. At high doses the carcinogenic potency can be derived directly from experimental data, but for risk levels of  $10^{-7}$  to  $10^{-5}$ , which correspond to very low doses, the  $q_1^*$  value must be derived by extrapolation from epidemiological data or from high dose, short-term animal bioassays.

### 3.2 CARCINOGENIC POTENCY CALCULATED FROM HUMAN DATA

In human epidemiological studies, carcinogenic effect is expressed in terms of the relative risk [RR(X)] of a cohort of individuals at exposure X compared with the risk in the control group [PR(control)] (e.g., if the cancer risk in group A is five times greater than that of the control group, then  $RR(X) = 5$ ). In such cases the "excess" relative cancer risk is expressed as  $RR(X) - 1$ , and the actual numeric, or proportional, excess risk level [PR(X)] can be calculated:

$$PR(X) = [RR(X) - 1] \times PR(\text{control}).$$

Using the standard risk/dose equation

$$PR(X) = b \times X$$

and substituting for PR(X):

$$[RR(X) - 1] \times PR(\text{control}) = b \times X$$

or

$$b = \frac{[RR(X) - 1] \times PR(\text{control})}{X}$$

where b is equal to the carcinogenic potency or  $q_1^*$ .

### 3.3 CARCINOGENIC POTENCY CALCULATED FROM ANIMAL DATA

In the case of animal studies where different species, strains, and sexes may have been tested at different doses, routes of exposure, and exposure durations, any data sets used in calculating the health criteria must conform to certain standards:

1. The tumor incidence must be statistically significantly higher than the control for at least one test dose level and/or the tumor incidence rate must show a statistically significant trend with respect to dose level.
2. The data set giving the highest index of cancer potency ( $q_1^*$ ) should be selected unless the sample size is quite small and another data set with a similar dose-response relationship and larger sample size is available.
3. If two or more data sets are comparable in size and identical with respect to species, strain, sex, and tumor site, then the geometric mean of  $q_1^*$  from all data sets is used in the risk assessment.
4. If in the same study tumors occur at a significant frequency at more than one site, the cancer incidence is based on the number of animals having tumors at any one of those sites.

In order to make different data sets comparable, the EPA guidelines call for the following standardized procedures:

1. To establish equivalent doses between species, the exposures are normalized in terms of dose per day (m) per unit of body surface area. Because the surface area is proportional to the  $2/3$  power of the body weight (W), the daily exposure (X) can be expressed as:

$$X = \frac{m}{W^{2/3}}$$

2. If the dose (s) is given as mg per kg of body weight:

$$s = \frac{m}{W}$$

then

$$m = s \times W$$

and the equivalent daily exposure (X) would be

$$X = \frac{(s \times W)}{W^{2/3}}$$

or

$$X = s \times W^{1/3}$$

3. The dose must also be normalized to a lifetime average exposure. For a carcinogenic assay in which the average dose per day (in mg) is m, and the length of exposure is  $l_e$ , and the total length of the experiment is  $L_e$ , then the lifetime average exposure ( $X_m$ ) is

$$X_m = \frac{l_e \times m}{L_e \times W^{2/3}}$$

4. If the duration of the experiment ( $L_e$ ) is less than the natural life span (L) of the test animal, the value of  $q_1^*$  is increased by a factor of  $(L/L_e)^3$  to adjust for an age-specific increase in the cancer rate.
5. If the exposure is expressed as the dietary concentration of a substance (in ppm), then the dose per day (m) is

$$m = \text{ppm} \times F \times r$$

where F is the weight of the food eaten per day in kg, and r is the absorption fraction (which is generally assumed to be equal to 1). The weight of the food eaten per day can be expressed as a function of body weight

$$F = fW,$$

where f is a species-specific, empirically derived coefficient which adjusts for differences in F due to differences in the caloric content of each species diet (f is equal to 0.028 for a 70-kg man: 0.05 for a 0.35-kg rat: and 0.13 for a 0.03-kg mouse).

Substituting (ppm x F) for m and fW for F, the daily exposure (dose/surface area/day or  $m/W^{2/3}$ ) can be expressed as

$$X = \frac{\text{ppm} \times F}{W^{2/3}} = \frac{\text{ppm} \times f \times W}{W^{2/3}} = \text{ppm} \times f \times W^{1/3}$$

6. When exposure is via inhalation, calculation can be considered for two cases: (1) the substance is a water soluble gas or aerosol and is absorbed proportionally to the amount of air breathed in and (2) the substance is not very water soluble and absorption, after equilibrium is reached between the air and the body compartments, will be proportional to the metabolic rate which is proportional to rate of oxygen consumption, which, in turn, is a function of total body surface area.

### 3.4 EXTRAPOLATION FROM HIGH TO LOW DOSES

Once experimental data have been standardized in terms of exposure levels, they are incorporated into a mathematical model which allows for calculation of excess risk levels and carcinogenic potency at low doses by extrapolation from high dose situations. There are a number of mathematical models which can be used for this procedure (see Krewski et al. 1983 for review). The EPA has selected a "linearized multi-stage" extrapolation model for use in deriving water quality criteria (USEPA 1980). This model is derived from a standard "general product" time-to-response (tumor) model (Krewski et al. 1983):

$$P(t;d) = 1 - \exp(-g(d)H(t)),$$

where  $P(t;d)$  is the probable response for dose  $d$  and time  $t$ ,  $g(d)$  is the polynomial function defining the effect of dose level, and  $H(t)$  is the effect of time:

$$g(d) = \sum_{i=0}^a \alpha_i d^i$$

$$H(t) = \sum_{i=0}^b \beta_i t^i$$

(with  $\alpha$  and  $\beta \geq 0$ , and  $\sum \beta_i = 1$ ).

This time-to-response model can be converted to a quantal response model by incorporation of the time factor into each  $\alpha$  as a multiplicative constant (Crump 1980):

$$p(d/t) = 1 - \exp\left(-\sum_{i=0}^a \alpha_i d^i\right),$$

or as given in the EPA guidelines (USEPA 1980):

$$p(d) = 1 - \exp[-(q_0 + q_1 d + q_2 d^2 + \dots + q_k d^k)],$$

where  $P(d)$  is the lifetime risk (probability) of cancer at dose  $d$ .

For a given dose the excess cancer risk  $A(d)$  above the background rate  $P(0)$  is given by the equation:

$$A(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

where

$$A(d) = 1 - \exp[-q_1 d + q_2 d^2 + \dots + q_k d^k].$$

Point estimates of the coefficients  $q_1 \dots q_k$  and consequently the extra risk function  $A(d)$  at any given dose are calculated by using the statistical method of maximum likelihood. Whenever  $q_1$  is not equal to 0, at low doses the extra risk function  $A(d)$  has approximately the form:

$$A(d) = q_1 \times d.$$

Consequently,  $q_1 \times d$  represents a 95 percent upper confidence limit on the excess risk, and  $R/q_1$  represents a 95 percent lower confidence limit on the dose producing an excess risk of  $R$ . Thus  $A(d)$  and  $R$  will be a function of the maximum possible value of  $q_1$  which can be determined from the 95 percent upper confidence limits on  $q_1$ . This is accomplished by

using the computer program GLOBAL 79 developed by Crump and Watson (1979). In this procedure  $q_1^*$ , the 95 percent upper confidence limit, is calculated by increasing  $q_1$  to a value which, when incorporated into the log-likelihood function, results in a maximum value satisfying the equation:

$$2(L_0 - L_1) = 2.70554,$$

where  $L_0$  is the maximum value of the log-likelihood function.

Whenever the multistage model does not fit the data sufficiently, data at the highest dose are deleted and the model is refitted to the data. To determine whether the fit is acceptable, the chi-square statistic is used:

$$\chi^2 = \sum_{i=1}^h \frac{(X_i - N_i P_i)^2}{N_i P_i (1 - P_i)}$$

where  $N_i$  is the number of animals in the  $i$ th dose group,  $X_i$  is the number of animals in the  $i$ th dose group with a tumor response,  $P_i$  is the probability of a response in the  $i$ th dose group estimated by fitting the multistage model to the data, and  $h$  is the number of remaining groups.

The fit is determined to be unacceptable whenever chi-square ( $\chi^2$ ) is larger than the cumulative 99 percent point of the chi-square distribution with  $f$  degrees of freedom, where  $f$  equals the number of dose groups minus the number of nonzero multistage coefficients.

#### 4. HEALTH CRITERIA FOR NONCARCINOGENIC TOXIC SUBSTANCES

Water quality criteria that are based on noncarcinogenic human health effects can be derived from several sources of data. In all cases it is assumed that the magnitude of a toxic effect decreases as the exposure level decreases until a threshold point is reached at and below which the toxic effect will not occur regardless of the length of the exposure period. Water quality criteria (C) establish the concentration of a substance in ambient water which, when considered in relation to other sources of exposure [i.e., average daily consumption of nonaquatic organisms (DT) and daily inhalation (IN)], place the Acceptable Daily Intake (ADI) of the substance at a level below the toxicity threshold, thereby preventing adverse health effects:

$$C = \frac{ADI - (DT + IN)}{[2L + (0.0065 \text{ kg} \times BCF)]}$$

where 2L is the amount of water ingested per day, 0.0065 kg is the amount of fish and shellfish consumed per day, and BCF is the weighted average bioconcentration factor.

In terms of scientific validity, an accurate estimate of the ADI is the major factor in deriving a satisfactory water quality criterion.

The threshold exposure level, and thus the ADI, can be derived from either or both animal and human toxicity data.

#### 4.1 NONCARCINOGENIC HEALTH CRITERIA BASED ON ANIMAL TOXICITY DATA (ORAL)

For criteria derivation, toxicity is defined as any adverse effects which result in functional impairment and/or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (USEPA 1980).

A bioassay yielding information as to the highest chronic (90 days or more) exposure tolerated by the test animal without adverse effects (No-Observed-Adverse-Effect-Level or NOAEL) is equivalent to the toxicity threshold and can be used directly for criteria derivation. In addition to the NOAEL, other data points which can be obtained from toxicity testing are

- (1) NOEL - No-Observed-Effect-Level,
- (2) LOEL - Lowest-Observed-Effect-Level,
- (3) LOAEL - Lowest-Observed-Adverse-Effect-Level,
- (4) FEL - Frank-Effect-Level.

According to the EPA guidelines, only certain of these data points can be used for criteria derivation:

1. A single FEL value, without information on the other response levels, should not be used for criteria derivation because there is no way of knowing how far above the threshold it occurs.
2. A single NOEL value is also unsuitable because there is no way of determining how far below the threshold it occurs. If only multiple NOELs are available, the highest value should be used.
3. If an LOEL value alone is available, a judgement must be made as to whether the value actually corresponds to an NOAEL or an LOAEL.
4. If an LOAEL value is used for criteria derivation, it must be adjusted by a factor of 1 to 10 to make it approximately equivalent to the NOAEL and thus the toxicity threshold.

5. If for reasonably closely spaced doses only an NOEL and an LOAEL value of equal quality are available, the NOEL is used for criteria derivation.

The most reliable estimate of the toxicity threshold would be one obtained from a bioassay in which an NOEL, an NOAEL, an LOAEL, and a clearly defined FEL were observed in relatively closely spaced doses.

Regardless of which of the above data points is used to estimate the toxicity threshold, a judgement must be made as to whether the experimental data are of satisfactory quality and quantity to allow for a valid extrapolation for human exposure situations. Depending on whether the data are considered to be adequate or inadequate, the toxicity threshold is adjusted by a "safety factor" or "uncertainty factor" (NAS 1977). The "uncertainty factor" may range from 10 to 1000 according to the following general guidelines:

1. Uncertainty factor 10. Valid experimental results from studies on prolonged ingestion by man, with no indication of carcinogenicity.
2. Uncertainty factor 100. Data on chronic exposures in humans not available. Valid results of long-term feeding studies on experimental animals, or in the absence of human studies, valid animal studies on one or more species. No indication of carcinogenicity.
3. Uncertainty factor 1000. No long-term or acute exposure data for humans. Scanty results on experimental animals, with no indication of carcinogenicity.

Uncertainty factors which fall between the categories described above should be selected on the basis of a logarithmic scale (e.g., 33 being halfway between 10 and 100).

The phrase "no indication of carcinogenicity" means that carcinogenicity data from animal experimental studies or human epidemiology are not available. Data from short-term carcinogenicity screening tests may be reported, but they are not used in criteria derivation or for ruling out the uncertainty factor approach.

#### 4.2 CRITERIA BASED ON INHALATION EXPOSURES

In the absence of oral toxicity data, water quality criteria for a substance can be derived from threshold limit values (TLVs) established by the American Conference of Governmental and Industrial Hygienists (ACGIH), the Occupational Safety and Health Administration (OSHA), or the National Institute for Occupational Safety and Health (NIOSH), or from laboratory studies evaluating the inhalation toxicity of the substance in experimental animals. TLVs represent 8-hr time-weighted averages of concentrations in air designed to protect workers from various adverse



health effects during a normal working career. To the extent that TLVs are based on sound toxicological evaluations and have been protective in the work situation, they provide helpful information for deriving water quality criteria. However, each TLV must be examined to decide if the data it is based on can be used for calculating a water quality criterion (using the uncertainty factor approach). Also the history of each TLV should be examined to assess the extent to which it has resulted in worker safety. With each TLV, the types of effects against which it is designed to protect are examined in terms of its relevance to exposure from water. It must be shown that the chemical is not a localized irritant and there is no significant effect at the portal of entry, regardless of the exposure route.

The most important factor in using inhalation data is in determining equivalent dose/response relationships for oral exposures. Estimates of equivalent doses can be based upon (1) available pharmacokinetic data for oral and inhalation routes, (2) measurements of absorption efficiency from ingested or inhaled chemicals, or (3) comparative excretion data when associated metabolic pathways are equivalent to those following oral ingestion or inhalation. The use of pharmacokinetic models is the preferred method for converting from inhalation to equivalent oral doses.

In the absence of pharmacokinetic data, TLVs and absorption efficiency measurements can be used to calculate an ADI value by means of the Stokinger and Woodward (1958) model:

$$ADI = \frac{TLV \times BR \times DE \times d \times A_A}{(A_O \times SF)}$$

where

- BR - daily air intake (assume 10 m<sup>3</sup>),
- DE - duration of exposure in hours per day,
- d - 5 days/7 days,
- A<sub>A</sub> - efficiency of absorption from air,
- A<sub>O</sub> - efficiency of absorption from oral exposure, and
- SF - safety factor.

For deriving an ADI from animal inhalation toxicity data, the equation is:

$$ADI = \frac{C_A \times D_E \times d \times A_A \times BR \times 70 \text{ kg}}{(BW_A \times A_O \times SF)}$$

where

- C<sub>A</sub> - concentration in air (mg/m<sup>3</sup>),
- D<sub>E</sub> - duration of exposure (hr/day),
- d - number of days exposed/number of days observed,
- A<sub>A</sub> - efficiency of absorption from air,

- BR - volume of air breathed (m<sup>3</sup>/day),
- 70 kg - standard human body weight,
- BWA - body weight of experimental animals (kg),
- AQ - efficiency of absorption from oral exposure, and
- SF - safety factor.

The safety factors used in the above equations are intended to account for species variability. Consequently, the mg/surface area/day conversion factor is not used in this methodology.

## 5. ORGANOLEPTIC CRITERIA

Organoleptic criteria define concentrations of substances which impart undesirable taste and/or odor to water. Organoleptic criteria are based on aesthetic qualities alone and not on toxicological data, and therefore have no direct relationship to potential adverse human health effects. However, sufficiently intense organoleptic effects may, under some circumstances, result in depressed fluid intake which, in turn, might aggravate a variety of functional diseases (i.e., kidney and circulatory diseases).

For comparison purposes, both organoleptic criteria and human health effects criteria can be derived for a given water pollutant; however, it should be explicitly stated in the criteria document that the organoleptic criteria have no demonstrated relationship to potential adverse human health effects.

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